

U.S.S.N. 10/763,883
Filed: January 23, 2004
SECOND PRELIMINARY AMENDMENT

In the claims

Please amend the claims as follows:

1. (previously presented) An isolated nucleic acid molecule, comprising a nucleic acid sequence comprising at least 50 nucleotides of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:1 or 2, and nucleic acid sequences which under stringent conditions hybridize with SEQ ID NO:1 or 2.
2. (previously presented) The nucleic acid molecule according to claim 1, wherein said nucleic acid sequence comprises at least 50 nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 3, 5, 7, 9, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36, nucleic acid sequences which correspond to these nucleic acid sequences within the degeneration of the genetic code, and nucleic acid sequences which under stringent conditions hybridize with these sequences.
3. (previously presented) The nucleic acid molecule according to claim 2, wherein said nucleic acid sequence comprises the entire sequence.
4. (currently amended) The nucleic acid molecule according to claim 1, wherein at least one coding region is functionally deleted.
5. (previously presented) The nucleic acid molecule according to claim 1, having inserted therein at least one insertion cassette for transposon or phage mediated insertion.

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6. (previously presented) The nucleic acid molecule according to claim 1, further comprising at least one heterologous nucleic acid molecule coding for a polypeptide or peptide is inserted or deletion-inserted.

7. (previously presented) The nucleic acid molecule according to claim further comprising the sequences flanking said heterologous nucleic acid molecule each having a length of at least 50 nucleotides, preferred 200 - 250 nucleotides.

8. (original) The nucleic acid molecule according to claim 6 or 7, wherein said heterologous nucleic acid molecule comprises a nucleic acid sequence coding for a bacterial or viral antigen or homologue thereof.

9. (previously presented) The nucleic acid molecule according to claim 6, wherein said heterologous nucleic acid molecule comprises a nucleic acid sequence coding for a tumor antigen.

10. (previously presented) The nucleic acid molecule according to claim 7, wherein said heterologous nucleic acid molecule comprises at least one gene expression cassette.

11. (previously presented) The nucleic acid molecule according to claim 7, wherein said heterologous nucleic acid molecule comprises at least one transactivator cassette, selective marker cassette, invertase cassette or combination thereof.

12. (previously presented) The nucleic acid molecule according to claim 7, wherein said heterologous nucleic acid molecule comprises at least one nucleic acid sequence coding for a polypeptide or peptide targeting and/or immunostimulatory domain.

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13. (previously presented) A recombinant vector comprising the nucleic acid molecule according to claim 1.

14. (previously presented) A cell comprising the nucleic acid molecule according to claim 5.

15. (original) The cell according to claim 14, wherein the cell is a gram-negative cell.

16. (original) The cell according to claim 14, wherein the cell is a Salmonella cell.

17-21. (canceled)

22. (original) An attenuated gram-negative cell comprising the SPI2 gene locus, wherein at least one gene of the SPI2 locus is inactivated, wherein said inactivation results in an attenuation/reduction of virulence compared to the wild type of said cell.

23. (previously presented) The attenuated gram-negative cell according to claim 22, wherein at least one inactivated gene is selected from the group consisting of effector (sse) genes, secretion apparatus (ssa) genes, chaperon (ssc) genes and regulation (ssr) genes.

24. (previously presented) The attenuated gram-negative cell according to claim 22, wherein said cell is an Enterobacteriaceae cell.

25. (previously presented) The attenuated gram-negative cell according to claim 24, wherein said cell is selected from the group consisting of a Salmonella cell, a Shigella cell and a Vibrio cell.

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26. (previously presented) The attenuated gram-negative cell according to claim 24, wherein said cell has a broad host range.

27. (original) The attenuated gram-negative cell according to claim 26, wherein said cell is a Salmonella serotype Typhimurium Definitive Type 104 (DT104) cell.

28. (previously presented) The cell according to claim 22, wherein at least one inactivated gene is selected from the group consisting of sse, ssc and ssr.

29. (previously presented) The cell according to claim 22, wherein at least one inactivated gene comprises at least one sse gene.

30. (previously presented) The cell according to claim 29, wherein at least one sse gene is selected from the group consisting of sseC, sseD and sseE.

31. (previously presented) The cell according to claim 22, wherein at least one inactivated gene comprises at least one ssr gene.

32. (original) The cell according to claim 31, wherein said at least one ssr gene is ssrB.

33. (previously presented) The cell according to claim 22, wherein at least one inactivated gene comprises at least one ssc gene.

34. (original) The cell according to claim 33, wherein said at least one ssc gene is sscB.

35. (previously presented) The cell according to claim 22, wherein at least one gene is inactivated by a mutation comprising a deletion.

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36. (original) The cell according to claim 35, wherein said deletion comprises at least 6 nucleotides.
37. (previously presented) The cell according to claim 35, wherein the mutation comprises a deletion of the complete coding sequence for said gene.
38. (previously presented) The cell according to claim 22, wherein at least one gene is inactivated by a mutation comprising the insertion of a heterologous nucleic acid molecule.
39. (previously presented) The cell according to claim 35, wherein said mutation is a non-polar mutation.
40. (previously presented) The cell according to claim 22, wherein at least one additional gene located outside of the SPI2 locus is inactivated, wherein the inactivation results in a further attenuation/reduction of virulence compared to the wild type.
41. (original) The cell according to claim 40, wherein said additional gene comprises an aro gene.
42. (original) The cell according to claim 41, wherein said aro gene is aro A.
43. (original) The cell according to claim 40, wherein said additional gene is superoxide dismutase.
44. (previously presented) The cell according to claim 22, comprising at least one selective marker cassette.

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45. (original) The cell according to claim 44, wherein said selective marker cassette is capable of conferring an antibiotic resistance to the cell.

46. (previously presented) The cell according to claim 22 comprising at least one gene expression cassette.

47. (previously presented) The cell according to claim 22 comprising at least one transactivator cassette.

48. (previously presented) The cell according to claim 22 comprising at least one invertase cassette.

49. (previously presented) The cell according to claim 22 further comprising at least one insertion cassette.

50-68. (canceled)

69. (original) An attenuated gram-negative cell comprising the SPI2 gene locus, characterized by a lack of at least one SPI2 polypeptide, wherein said lack results in an attenuation/reduction of virulence compared to the wild type of said cell.

70. (previously presented) The attenuated gram-negative cell according to claim 69, wherein said missing polypeptide is selected from the group consisting of effector (sse) polypeptides, secretion apparatus (ssa) polypeptides, chaperon (ssc) polypeptides 68 and regulatory (ssr) polypeptides.

71-90. (canceled)

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91. (currently amended) An isolated nucleic acid molecule comprising a nucleic acid of at least 100 nucleotides of a sequence selected from the group consisting of SEQ ID NOS: 3, 5, 7, 9, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36, nucleic acid sequences which under stringent conditions hybridize with these sequences.

92. (original) The nucleic acid molecule according to claim 91, wherein said nucleic acid molecule is capable of inducing the expression of a nucleic acid sequence coding for a peptide or polypeptide operatively linked to said nucleic acid molecule.

93. (previously presented) Expression system for the *in vivo* inducible expression of a heterologous nucleic acid in a target cell, comprising a carrier cell for said heterologous nucleic acid, wherein said carrier cell comprises (a) a polypeptide having the amino acid sequence shown in SEQ ID NO:35 (ssrA) or a functional homologue thereof, (b) a polypeptide having the amino acid sequence shown in SEQ ID NO:37 (ssrB) or a functional homologue thereof, and (c) the nucleic acid molecule according to claim 92.

94. (original) Expression system according to claim 91, wherein said target cell is a macrophage.

95. (previously presented) Expression system according to claim 93, wherein said carrier cell is a Salmonella cell.

96. (previously presented) The expression system according to claim 93, wherein said target cell comprises a gene expression cassette.

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97. (previously presented) The expression system according to claim 93, wherein said target cell comprises an insertion cassette.

98. (previously presented) The expression system according to claim 93, wherein said target cell comprises a heterologous nucleic acid molecule coding for a peptide or polypeptide.

99-100. (canceled)